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ADAPTATION BY MICROBIAL POPULATIONS

Norman C. Dondero, et al

Cornell University
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PROGRESS REPORT

For the Period September 1, 1967 to May 31, 1968

ADAPTATION BY MICROBIAL POPULATIONS

Norman C. Dondero, Principal Investigator

T. B. S. Prakasam, Research Associate

Department of Food Science
Cornell University
Ithaca, New York 14850

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Previous progress reports dealt with the enumeration and characterization of populations of the aerobic heterotrophs of sewage, activated sludge, and laboratory sludges acclimated to artifical "wastes". Much of the material has been incorporated into published reports (4, 5, 6, 7); two additional manuscripts are in preparation.

In addition to examining the heterotrophs of waste waters and treatment processes, the original objectives of the project included the examination of natural waters and polluted waters. This report contains material concerned with waste water treatment and with the waters of Cayuga Lake.

Waste Water Studies

In pursuance of the studies on the activated sludge extract media, experiments were undertaken on the effect of the quantity of the mixed liquor suspended solids used in making the activated sludge extract medium on the productivity.

The mixed liquor from the aeration tank of the Marlboro, New Jersey, sewage treatment plant, (MLSS: 2580 mg/l) was used in making activated sludge extracts of different strengths, as indicated below:

Extract Strength	MLSS Concentration mg/l
1/4 X	645
1/2 X	1290
2 X	5160

The double strength extract was made by settling 1 liter of the mixed liquor for 1/2 hour and discarding 500 ml of the supernatant. The remainder of the supernatant and the settled solids were autoclaved for 15 minutes at 121 C and filtered. Similarly 1/2X and 1/4X extracts were prepared by diluting the mixed liquor with distilled water to yield the desired strength.

Ionagar No. 2 was then added (0.85% w/v) to the extracts, which were sterilized once more for 15 minutes at 121 C.

Homogenized and serially diluted samples of activated sludge plated in quadruplicate on the above media and incubated at 20 C for 7 days yielded the following mean counts:

Table 1. Effect of medium strength on productivity.

Strength of ASE medium	Viable plate counts (10^6/ ml.)
1/4 X	116
1/2 X	111
2 X	105

Analysis of variance (Table 2) of the transformed plate count data (transformation procedure discussed in a previous progress report) indicated that there were no significant differences in the productivity of the media, indicating that the strength of the extract was not related to the productivity within the range tested. However, previous experience in the case of one experimental run showed that a double strength extract medium suppressed colony counts.

Table 2. Analysis of variance of the plate count data.

		df	MSS
Total sum of squares	1679.00	15	
Correction factor	1668.06	1	
Total corrected sum of squares	10.94	14	
within media	0.86	2	0.43
Error	10.08	12	0.84

In another experiment, from the mixed liquor of the Marlboro treatment plant (MLSS, 3455 mg/l), we made extracts of the following strengths: 1X, 1/2X, 2/5X, 1/5X; to give MLSS concentrations of 3455, 1728, 1382, and 691 mg/l, respectively. Samples of Marlboro activated sludge were plated on the media of the above strengths. Table 3 indicates the results obtained.

Table 3. Effect of the strength of the ASE medium on productivity.

Strength of the ASE medium	Colony counts (10^6/ml)
1X	277
1/2X	243
2/5X	245
1/5X	257

Statistical analysis of the above data also indicated that the strength of the medium, within the range tested, had no effect on the productivity.

In conjunction with the above experiment, activated sludge extracts were made using 691, 1382, and 6910 mg/l of mixed liquor suspended solids from Bernardsville treatment plant to represent the media strengths equivalent to 1/5X, 2/5X, and 2X of the Marlboro activated sludge extracts used in the previous experiment. The plating of Bernardsville, New Jersey, activated sludge was performed on these media. Quadruplicate plates were set for incubation at 20 C for 7 days. The following were the results obtained.

Table 4. Effect of the strength of medium on productivity.

Strength of ASE (Ber) medium in equivalents of ASE (Mar)	Mean viable counts 10^6/ml
1/5X	229
2/5X	175
2X	199

Analysis of Variance of the plate count data.

		df	MSS
Total sum of squares	3028	15	
Correction factor	2989.2	1	
Total corrected sum of squares	38.8	14	
Media	8.52	2	4.26
Error	30.28	12	2.52

From the above data and its analysis of variance it can be judged that the means are not significantly different. The results are thus in agreement with the previous results and also indicated that activated sludge extracts could be made from the mixed liquor of either treatment plant within the tested range of strengths for the enumeration of activated sludge bacteria. This conclusion is based on the results obtained from 2 treatment plants primarily treating domestic wastes and it may not apply to sludges from plants treating certain industrial wastes.

BACTERIOLOGY OF MARLBORO SEWAGE TREATMENT PLANT.

A study was undertaken to enumerate and classify by Lochhead's nutritional system, the bacteria in the settled sewage, mixed liquor from the inlet and outlet ends of the aeration tank, returned sludge, unchlorinated effluent and chlorinated effluent at the Marlboro, New Jersey, treatment plant. Samples were simultaneously collected, and plated on ASE medium. Master

plates for the Lochhead series were also made, but were lost through a laboratory mishap. The plates were incubated for 10 days at 20 C. The following were the results obtained.

Table 5. Bacteriological data of Marlboro sewage treatment plant.

Sample	Strength of ASE medium	Mean viable counts per ml.
Settled sewage	1X	8.25×10^6
Mixed liquor from the inlet end of aeration tank	1/2X	250×10^6
Mixed liquor from the outlet end of aeration tank	1/2X	284×10^6
Returned sludge	1/2X	1000×10^6
Unchlorinated effluent	1X	1.25×10^6
Chlorinated effluent	1X	2400.

An over-all reduction of 85% of the bacteria was accomplished in the activated sludge process without chlorination. The bacterial removal was 99.9% with chlorination. Chlorination alone killed 99.9% of the bacteria contained in the secondary effluent.

SUPPLEMENTAL EXPERIMENTS OF NUTRITIONAL CHARACTERIZATION OF ACTIVATED SLUDGE BACTERIA:

In the progress report for the year ending August 1966, we reported on the nutritional characterization of activated sludge bacteria using the Lochhead's technique.(3) In the series of media used, the "basal medium" had the following composition: glucose, 1g; K_2HPO_4, 0.5g; KNO_3, 0.5g; $MgSO_4$, 0.2 g; $CaCl_2$, 0.1 g, NaCl, 0.1 g; distilled water, 1 liter. In a recent paper, Sundman and Calberg(9) reported that the use of glucose and inorganic nitrogen in the basal medium of Lochhead excluded soil organisms with simple and nutritional demands to the extent of about 17% of the total

count. They recommended the replacement of glucose with lactate or augmentation with lactate, glycerol, acetate, and citrate. In light of these recommendations, a plating comparison was made with samples from various points of the Marlboro sewage treatment plant on the basal medium of Lochhead and on a slightly modified Sundman and Calberg medium. The modified medium of Sundman and Calberg had the following composition:

$(NH_4)_2$ H PO_4	0.3267 g
K_2HPO_4	0.0689 g
glucose	0.2 g
Sod. acetate	0.2732 g
$MgSO_4$	0.2 g
$CaCl_2$	0.1 g
NaCl	0.1 g
Sod. Citrate	0.3868 g
Sod. lactate	0.2075 g
Glycerol	0.2044 g
Dist. water	1 liter

Samples of settled sewage, mixed liquor from the aeration tank and the unchlorinated effluent of Marlboro sewage treatment plant were plated on the two basal media with the following results (Table 6):

Table 6. Comparison of viable counts on two basal media. (10^6/ml)

Sample	Basal medium (Lochhead)	Basal medium Modified Sundman and Calberg
Settled sewage	4.3	2.7
Mixed liquor	220	165
Unchlorinated effluent	0.43	0.38

Statistical analysis of the data indicated that, with the exception of the unchlorinated effluent, the basal medium of Lochhead gave significantly higher counts than the modified Sundman and Calberg medium. Thus it appears that there was no exclusion of activated sludge bacteria having simple nutritional requirements by the Lochhead basal medium, or that there were not significant numbers of such microorganisms, as observed by Sundman and Calberg;

TRICKLING FILTER BACTERIA AND THEIR NUTRITIONAL CHARACTERIZATION:

In exploratory experiments, the productivity of the trickling filter slime extract (TFSE) was compared to that of ASE. The TFSE was made by autoclaving one liter of trickling filter slime (the sloughed-off material from the filter collected before it is pumped to the digester) at 121 C for 15 minutes and by filtering through glass wool. The filtrate was made up to a liter with distilled water, Ionagar No. 2 added (0.85% w/v), and then autoclaved for 15 minutes at 121 C. Activated sludge extract made from the Bernardsville, New Jersey, treatment plant was used for comparison.

For viable plate counts, the trickling filter slime was homogenized, serially diluted, and plated on the TFSE and ASE agars. The following were the results obtained (Table 7).

Table 7. Viable counts of Trickling Filter Slime.

Medium	Viable counts, (10^6/ml)
TSEF	2061
ASE	2237

Statistical analysis of the above plate count data showed that there was no significant difference in the productivity. However, the colonies on TSEF were big and moist, whereas those on ASE were small.

The trickling filter bacteria were characterized nutritionally by Lochhead's system done in the manner as for activated sludge bacteria. Bacterial colonies developed on either TFSE or ASE master plates were transferred by replica plating to the following media:

1. Lochhead basal medium (BM)
2. B.M. + yeast extract
3. B.M. + yeast extract + vitamin B_{12}
4. B.M. + amino acids
5. B.M. + growth factors
6. B.M. + growth factors + amino acids

The master plates and the replica plates were incubated at 20 C for 10 days with the following results.

Table 8. Nutritional characterization of Trickling filter bacteria.

	VIABLE COUNTS (10^6/ml)						
Master plate	Basal medium	BM+YE	BM+YE+ B_{12}	BM + Amino Acids	BM + growth factors	BM + Amino acids+ growth factors	Number of colonies that did not grow on any media.
1. No. of Colonies:74	44	64	66	64	58	71	3
2. No. of Colonies:54	38	45	48	43	40	45	6
3. No. of Colonies:37	23	28	29	27	28	29	8
4. No. of Colonies:25	16	19	17	18	18	18	6
Total: 190	121	156	160	152	144	163	23

From the above data it can be seen that on an average 10 per cent of the trickling filter bacteria did not grow even on the most complex medium used in this study. About 63.4% of the bacteria have simple nutritional requirements, as indicated by their growth on the basal medium containing only glucose and salts. About 20% needed yeast extract for their growth while an additional 2% grew on the yeast extract medium supplemented with Vitamin B_{12}.

Of the total, 80% of the bacteria were able to grow on the basal medium supplemented with casamino acids indicating that 15.6% of the population needed amino acids for their growth. The addition of growth factors to the amino acids medium resulted in an additional recovery of 5.7% $(\frac{163-152}{190} \times 100)$ of the total bacterial colonies.

The addition of growth factors to the basal medium had a stimulatory effect and the recovery of colonies was 12% more than on the basal medium alone.

It appeared that many of the organisms could substitute yeast extract for amino acids and growth factors. A similar observation was made with activated sludge bacteria.(5)

MICROBIOLOGY OF LAKE WATERS:

Preliminary studies were undertaken to enumerate and study the behavior of the microorganisms found in the water of Cayuga Lake. Cayuga Lake is a natural lake formed by glaciation, but the level is maintained by locks at the northern end. It has an elevation of 381' and a surface area of 66.4 sq. miles. The mean depth of the lake is 179 feet with a maximum of 435 feet. The southern end of the lake receives chlorinated effluent from the city of Ithaca and the village of Cayuga Heights. The Ithaca treatment plant is a trickling filter plant; Cayuga Heights provides only primary treatment.

The chemical analysis of the water on March 11, 1968, was as follows:

Temperature:	3° C
pH:	8
D.O.	12-13 mg/l
Alkalinity (Phenolphthalein)	0
Total alkalinity as $CaCO_3$	105 mg/l
Cl^- as Cl	87.5 mg/l
Ca^{++} hardness as $CaCO_3$	100 mg/l
Total hardness "	140 mg/l
Mg hardness "	40 mg/l

ENUMERATION OF LAKE WATER BACTERIA:

Lake water was collected and continuously pumped from an intake source with 80 feet from shore and 80 feet deep. Upon arrival at the laboratory, 160 liters of the sample is centrifuged in a Sharples centrifuge. The sediment is collected from the bowl and suspended in 200 ml of lake water to give a concentration ratio of 800:1. The suspension is gently stirred on a magnetic stirrer until a homogeneous suspension is attained, serially diluted in sterile distilled water, and plated on various media. The following media were tested in enumeration studies:

1. Lake water agar (LWA)
2. Iron peptone medium (IP)
3. "Gravel extract" (GE)
4. Activated sludge extract (ASE)
5. Taylor's medium (TM)
6. Hayes-Anthony medium (HA)
7. Nutrient agar (NA)

8. Casitone-glycerol-yeast autolysate medium (CGY)

9. Casitone-yeast autolysate (CY)

LAKE WATER AGAR:

To one liter of lake water, 8.5 g of Ionagar No. 2 was added before sterilization at 121 C for 15 minutes.

IRON PEPTONE MEDIUM: (1)

Proteose peptone, 5 g; ferrous sulfate, 154 mg; sodium gluconate, 90 mg; ammonium sulfate, 100 mg; tap water, 1000 ml. pH 7.0.

"GRAVEL EXTRACT":

One kilogram of gravel and adherent material collected from the lake shore was added to one liter of distilled water and autoclaved for 15 minutes at 121 C. After filtration through glass wool, the filtrate was made up to one liter with distilled water.

ACTIVATED SLUDGE EXTRACT:

One liter of mixed liquor from the effluent end of the aeration tank was autoclaved for 30 minutes at 121 C. The glass wool filtrate was made up to one liter with distilled water, Ionagar No. 2 added (0.85% w/v), and was autoclaved again for 30 minutes.

TAYLOR'S MEDIUM:

Peptone, 0.5 g; sodium caseinate, 0.5 g; soluble starch, 0.5 g; glycerol, 1.0 g; K_2HPO_4, 0.2 g; $MgSO_4$, 0.05 g; $FeCl_3$, trace; Ionagar No. 2, 8.5 g; distilled water, 1000 ml.

HAYES-ANTHONY MEDIUM: (2)

Glycerol, 1 g; sodium caseinate, 0.5 g; peptone, 0.5 g; starch, 0.5 g; liver extract, 0.5 g; $MgSO_4$, 0.05; $CaCl_2$, 0.05 g; K_2HPO_4, 0.2 g. Add KOH to bring pH after autoclaving to 7.0. After autoclaving, add vitamin B_{12} (7 ug) and a trace of iron ($FeCl_3$ or $FeSO_4$).

NUTRIENT AGAR:

Bacto nutrient agar was used.

CASITONE-GLYCEROL-YEAST AUTOLYSATE MEDIUM:

Casitone, 5 g; glycerol, 5 g; yeast autolysate, 1.5 g; distilled water, 1000 ml; Ionagar No. 2, 8.5 g.

CASITONE-YEAST AUTOLYSATE:

Casitone, 5 g; yeast autolysate, 1.5 g; distilled water, 1000 ml; Ionagar No. 2, 8.5 g.

STATISTICAL TREATMENT OF ENUMERATION DATA:

In the plating experiments the mean counts were derived from the counts of a minimum of three replicates. Since the bacterial counts followed the Poisson distribution, the plate count data was first subjected to square root transformation so that procedures designed for normally distributed populations could be used. This transformation involved taking the positive square root of each plate count (x). The means of plate counts reported in the results of plating experiments were obtained by squaring the arithmetic mean of all square roots of x, not by averaging raw plate counts as such. Analysis of variance was calculated on the square root transformed data. The least significant difference (lsd) derived from the transformed data was used to determine which differences between means were significant, and to provide a basis for grouping those means which could not be shown to be significantly different. In the following tables these similar means are underlined by bars.

PLATING EXPERIMENT I:

The first seven media mentioned above were used for enumeration of lake water bacteria. The following were the results obtained.

Table 10. Viable counts of Lake Water. Plating Experiment I.

No.	Medium	Mean viable counts (10^4 per ml of the concentrated sample (800:1)
1.	LWA	50
2.	IP	159
3.	GE	220
4.	ASE	785
5.	TAY	1085
6.	HA	678
7.	NA	177

Analysis of variance of the viable count data.

		df	MSS
Total sum of squares	10396	21	
Correction factor	8290.04	1	
Total corrected sum of squares	2095.96	20	
Media	2071.62	6	345
Error	24.34	14	1.7

The computed $F_{(6, 14)}$ is much higher than the tabular value of the F distribution for the same degrees of freedom and hence we can say that the means are significantly different from each other. The means were ranked by the lsd as shown in Table 11.

Table 11. Summary of ranking plate count media in order of effectiveness for lake water by lsd.

Medium	LWA	IP	NA	GE	HA	ASE	TAY
Counts x 10^4 (concentrated sample)	50	159	177	220	678	784	1085
Counts in lake water, per ml.	625	1990	2210	2750	8475	9800	13560

Of the culture media tested, Taylor's medium was the most productive for the lake water bacteria.

Plating Experiment II. In this experiment CGY and CY media were used instead of LWA and GE. Table 12 shows the results.

Table 12. Viable counts of Lake Water. Plating Experiment II.

No.	Medium	Mean viable counts x 10^4 per ml of the concentrated sample (800:1)
2.	IP	197
4.	ASE	196
5.	TAY	258
6.	HA	219
7.	NA	83
8.	CGY	114
9.	CY	127

Analysis of variance of the plate count data, Experiment II.

		df	MSS
Total sum of squares	3634	21	
Correction factor	386	1	
Total Corrected Sum of Squares	3248	20	
Media	3233.9	6	539
Error	14.1	14	1.006

From the analysis of variance it can be judged that the means are significantly different. Ranking by lsd showed the following:

Table 13. Summary of ranking plate counts in order of effectiveness of media for lake water bacteria by lsd.

Medium	NA	CGY	CY	ASE	IP	HA	TAY
Counts x 10^4 (concentrated sample)	83	114	127	196	197	219	258
Counts in lake water per ml.	104	1430	1567	2450	2463	2737	3225

The CGY and CY media were inferior to Taylor's medium which was again found to be the best of the assemblage of media for the enumeration of lake water bacteria. Nutrient agar, a very rich medium suitable for enumeration of sewage bacteria,[8] was found to be poor for the cultivation of lake water bacteria.

PSYCHROPHILIC ORGANISMS IN LAKE WATER:

Most of the water samples were between 2 to 6 C when collected. Hence, the enumeration of cold tolerant bacteria is of interest. In plating experiment I, a duplicate set of plates was spread with the concentrated lake water inoculum and incubated at 4.5 C for 15 days. The following were the counts obtained:

Table 14. Viable counts of lake water at 4.5 C.

No.	Medium	Mean viable counts x 10^4 per ml of concentrated sample (800:1)
1.	LWA	45
2.	IP	93
3.	GE	102
4.	ASE	396
5.	TAY	497
6.	HA	195
7.	NA	247

Analysis of variance of the viable count data.

		df	MSS
Total sum of squares	4782	21	
Correction factor	4139.66	1	
Total corrected sum of squares	642.34	20	
Media	583.99	6	97
Error	58.35	14	4.1

The analysis of variance indicates that the means are significantly different.

Ranking of the productivity of media by lsd indicates the following:

Table 15. Summary of ranking plate count media in order of effectiveness for lake water by lsd, Plating Experiment I, at 4.5 C.

Medium	LWA	IP	GE	HA	NA	ASE	TAY
Counts x 10^4 per ml of concentrated sample	45	93	102	195	247	396	497
Counts in lake water	562	1160	1280	2440	3100	4950	6210

For the enumeration of cold tolerant bacteria also, it appeared that Taylor's medium was superior to the other media, except for activated sludge extract. About 45% of the lake water bacteria grew at 4.5 C.

BIOCHEMICAL ACTIVITY OF THE LAKE WATER BACTERIA:

Experiments on attack of nutrient substrates by the mixed flora were initiated with a two-fold purpose: (1) to determine the compounds readily attacked and (2) to seek a basis for rational design of culture media.

The first provides clues as to the activity of the flora in nature and its possible adaptive reactions to foreign substances introduced into its environment and also allows comparison with the activities of soil and waste water bacteria. The second objective seeks insights into the best means of enumeration, enrichment, or isolation of the microflora and its components.

A variety of compounds, representing different chemical classes and different mixtures, including sewage, will be tested.

Oxygen uptake experiments have been performed with the following compounds:

1. Glycerol
2. Arabinose
3. Xylose
4. Ribose
5. Glucose
6. Mannose
7. Fructose
8. Galactose
9. Rhamnose
10. Sucrose
11. Maltose
12. Lactose
13. Invert sugar
14. Starch
15. Raffinose
16. Sod. acetate
17. Sod. lactate
18. Sod. citrate
19. Sod. succinate
20. α-keto glutaric acid
21. Sod. formate
22. Casitone
    a) Sod. caseinate
23. Peptone, bacto
24. Tryptone, bacto
25. Casamino acids
26. Alanine
27. Asparagine
28. Aspartic acid
29. Cystine
30. Cysteine
31. Glutamic acid
32. Glycine
33. Histidine
34. Isoleucine
35. Leucine
36. Lysine
37. Methionine
38. Phenylalanine

RESPIROMETRY:

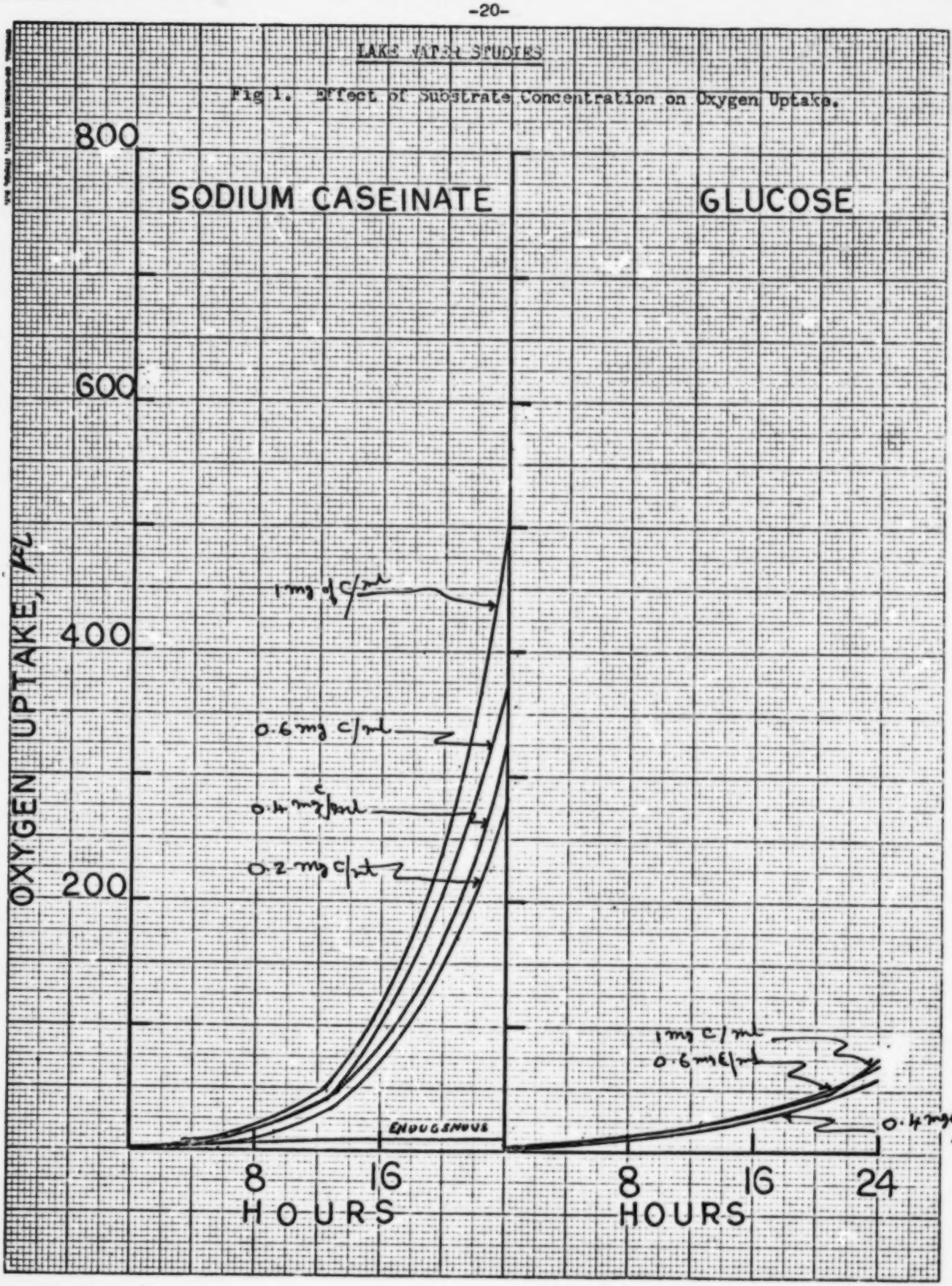
A Gilson differential respirometer was used in these studies. Fifteen ml flasks with side arm were used. The volume of contents in the flasks was: concentrated lake water suspension, 1 ml; 15% KOH (w/v), 0.2 ml in the central well; salts solution, (K_2HPO_4, 0.5 g; KH_2PO_4, 0.5 g; $MgSO_4$, 0.5 g; $(NH_4)_2\ SO_4$, 0.75 g; NaCl, 0.005 g; $FeCl_3$, 0.002 g; Mn SO_4, 0.001 g; $CaCl_2$, 0.005 g; and distilled water, 1000 ml) 1 ml. Substrates equivalent to 0.5 mg of C per ml were placed in the side arm of the flasks except as otherwise stated. The water bath temperature was maintained at 20 C. After equilibrating the flasks for 20 minutes, the substrates were tipped in.

In a series of preliminary tests, the effect of the substrate concentration on the oxygen uptake of lake water microorganisms was studied. The substrates used in this investigation were: 1. sod. caseinate, 2. glucose, 3. sodium acetate, and 4. casamino acids. The levels of C tested were: 1 mg, 0.6 mg, 0.4 mg, 0.2 mg, and 0.1 mg per ml. Figs 1 and 2 show the results obtained. Within the ranges of C tested, the 1 mg per ml exerted the maximum uptake. Sodium caseinate and casamino acids exerted very high oxygen uptakes although there was an initial lag for about eight to twelve hours. Glucose was a poor substrate for lake water microorganisms in comparison to acetate, casamino acids and sodium caseinate.

In another experiment, the effect of mixtures of materials likely to be stimulatory to activity such as sea salts, yeast extract and vitamin B_{12} on the oxygen uptake of lake water microorganisms on various substrates was studied. The substrate concentration was equivalent to 0.5 mg of C per ml of the flask contents. The substrates tested were: (1) sodium caseinate, (2) sodium acetate, (3) glucose, and (4) sodium succinate. The amount of yeast extract and vitamin B_{12} used was 0.05% (w/v) and 5 ug per liter respectively.

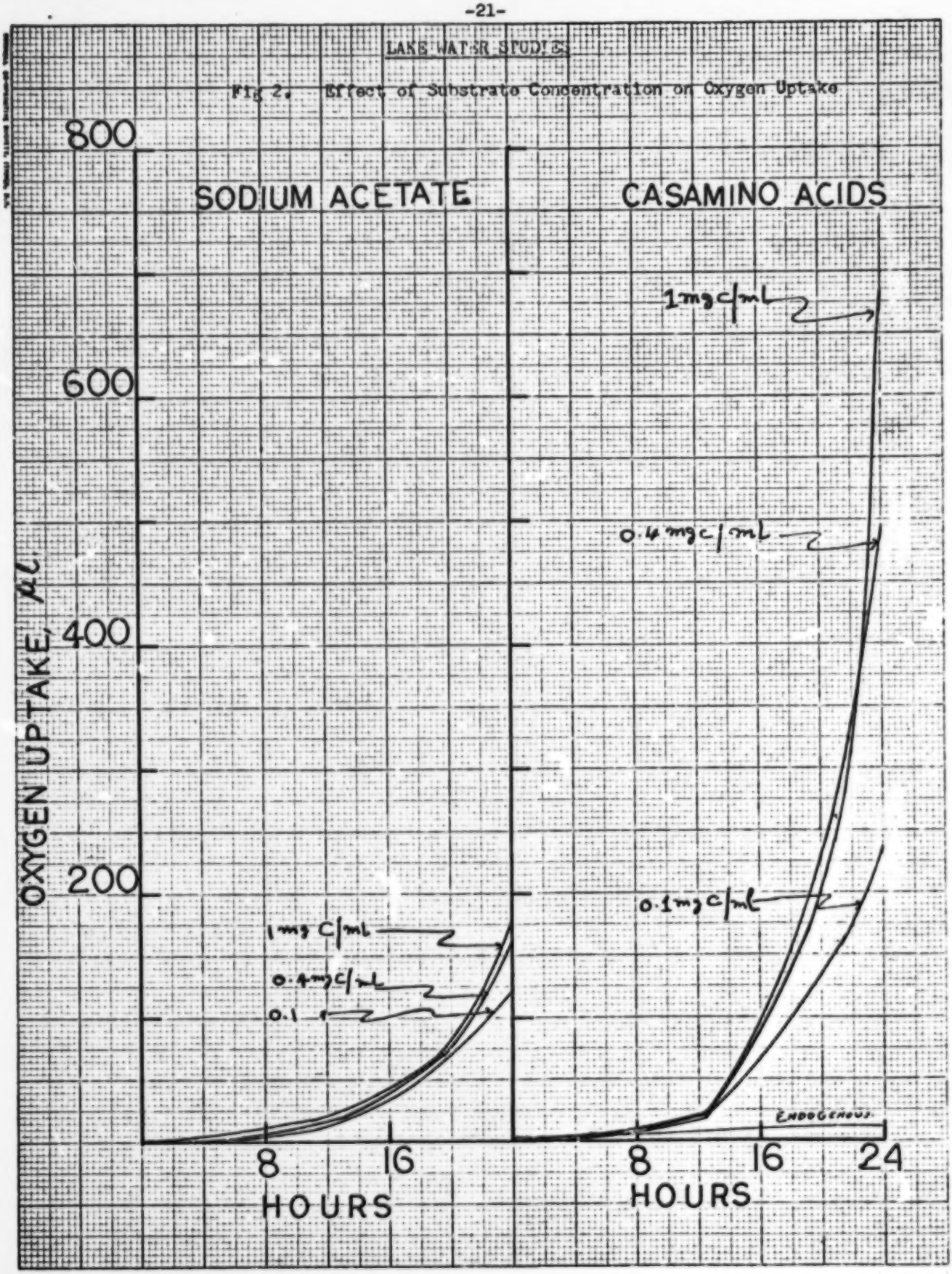
LAKE WATER STUDIES

Fig 1. Effect of Substrate Concentration on Oxygen Uptake.



LAKE WATER STUDIES

Fig 2. Effect of Substrate Concentration on Oxygen Uptake



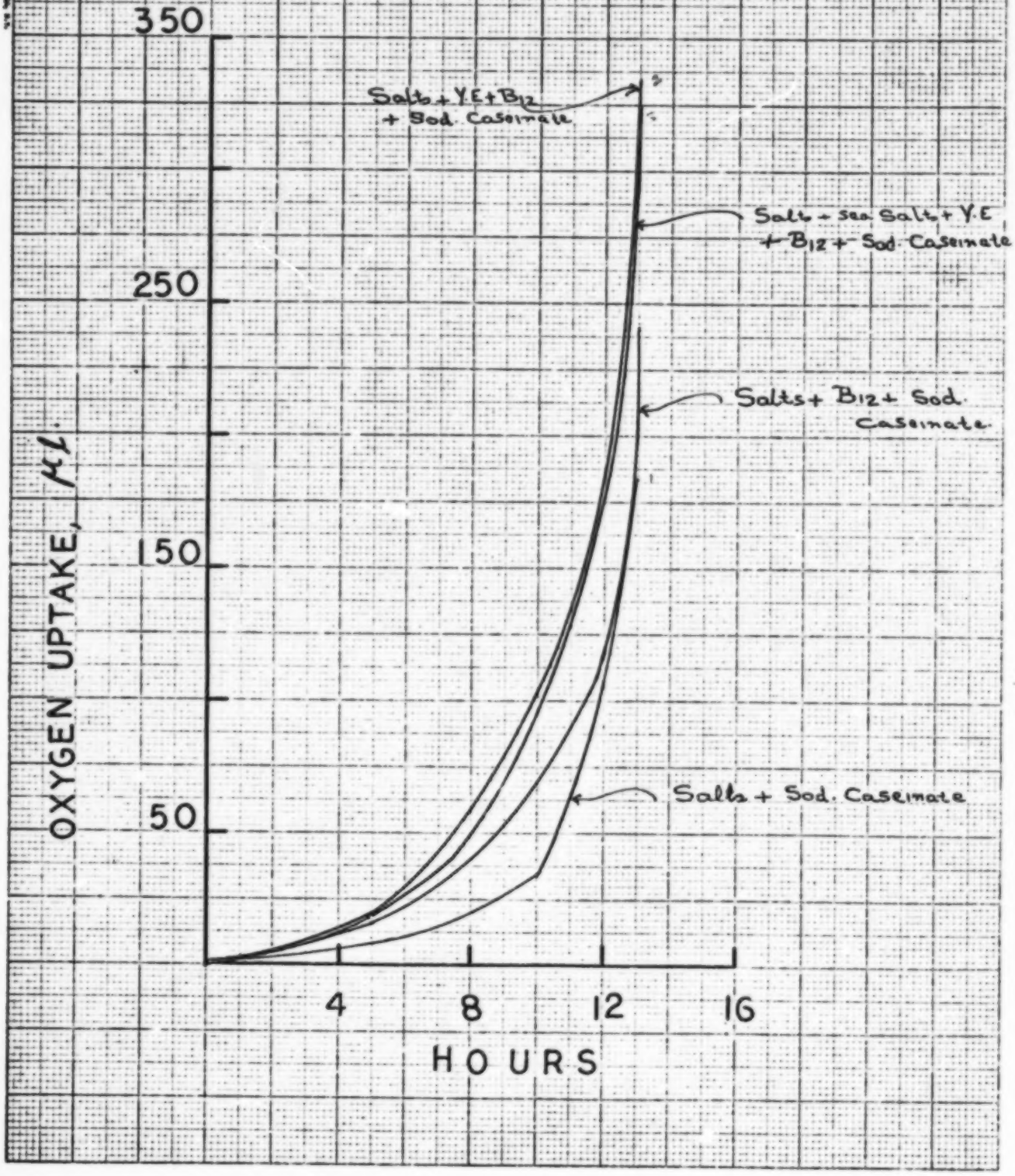
0.5 ml of 0.01% sea salts solution and 0.5 ml of the salts solution previously described were also added to the flask contents.

Figs. 3, 4, 5, and 6 represent the results. Sea salts did not seem to stimulate except with sodium acetate. Vitamin B_{12} stimulated the oxygen uptake with caseinate and acetate, but had no effect with glucose and succinate. The addition of yeast extract and yeast extract plus vitamin B_{12} increased the oxygen uptake with all the substrates tested, but it was not clear whether this was a stimulatory effect or an increase in uptake due to attack on the yeast extract itself. In an experiment to clarify the point, yeast extract was used at different levels, covering a range of 0 to 1000 mg per liter with glucose maintained at 0.5 mg of C per ml.

Figure 7 indicates the results obtained with yeast extract. It is clear that oxygen uptake was attributed only to consumption of yeast extract. On the other hand, examination of the graphs for salts + yeast extract (500 mg per liter) and salts + glucose + yeast extract (500 mg per liter) indicate that glucose suppressed somewhat the oxygen uptake exerted by the yeast extract.

LAKE WATER STUDIES

Fig. 3. Effect of sea salts, yeast extract, vit. B_{12} on the Oxygen Uptake.



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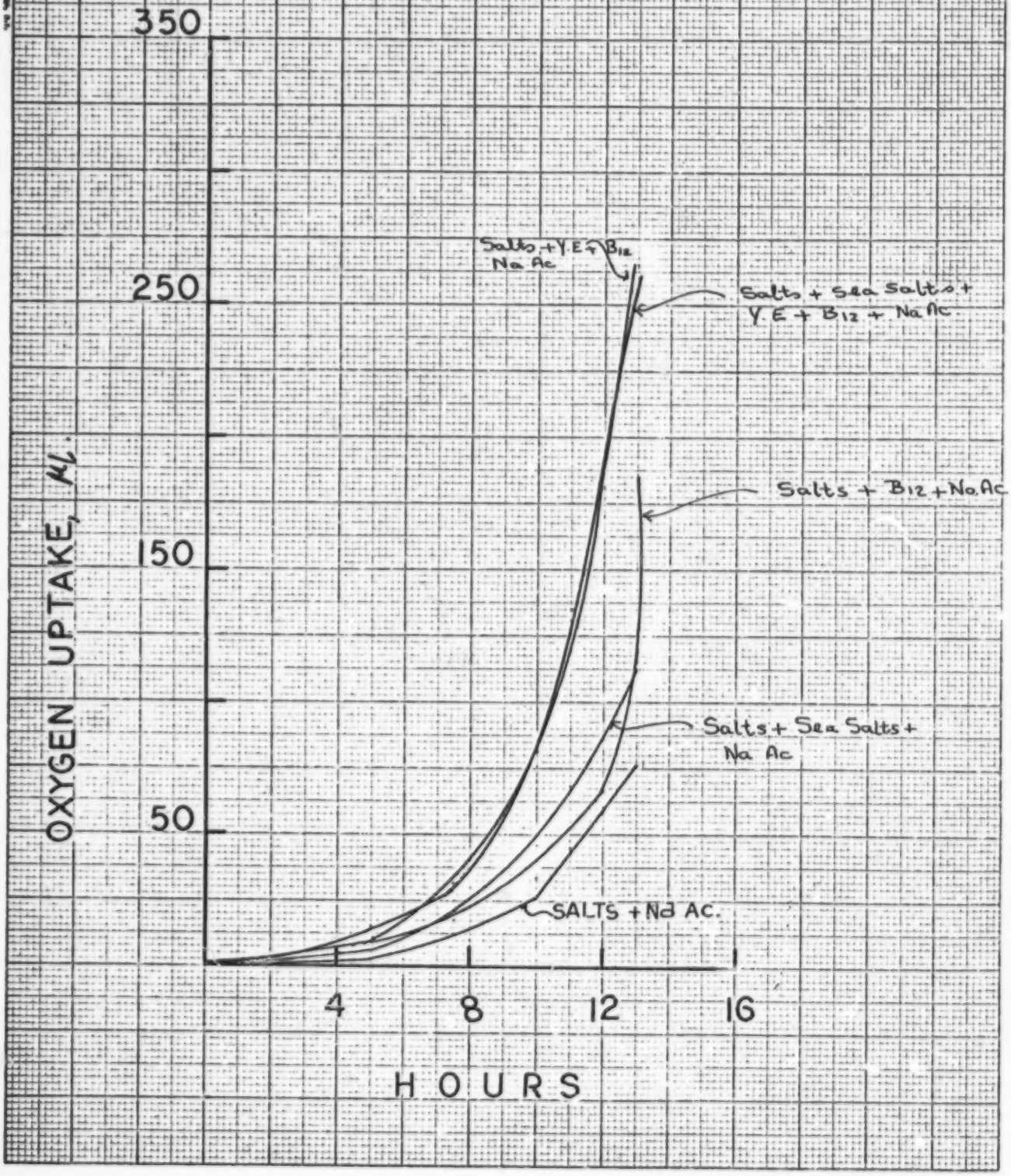
LAKE WATER STUDIES

Fig. 4. Effect of sea salts, yeast extract, and vit B_{12} on the Oxygen Uptake.



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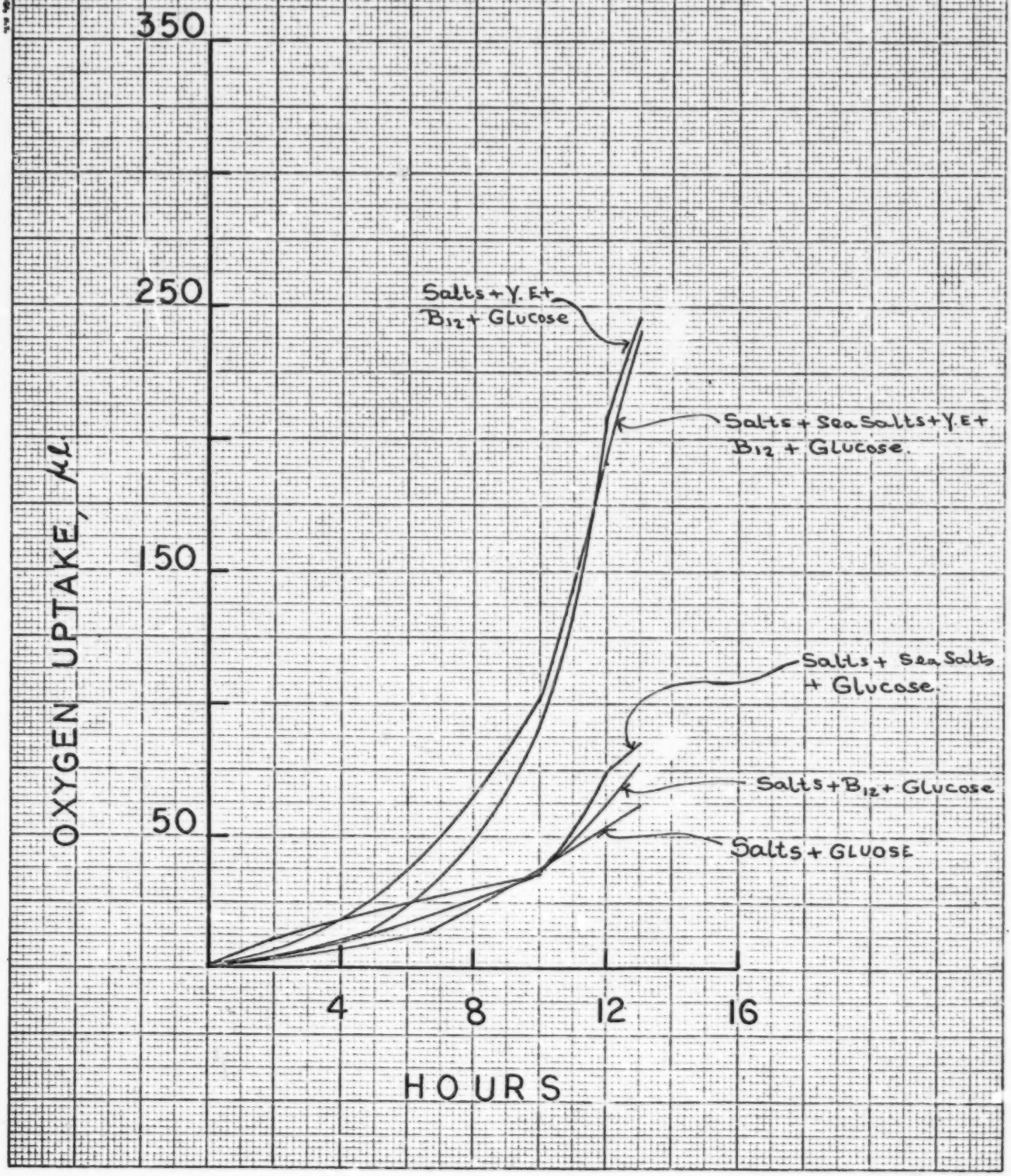
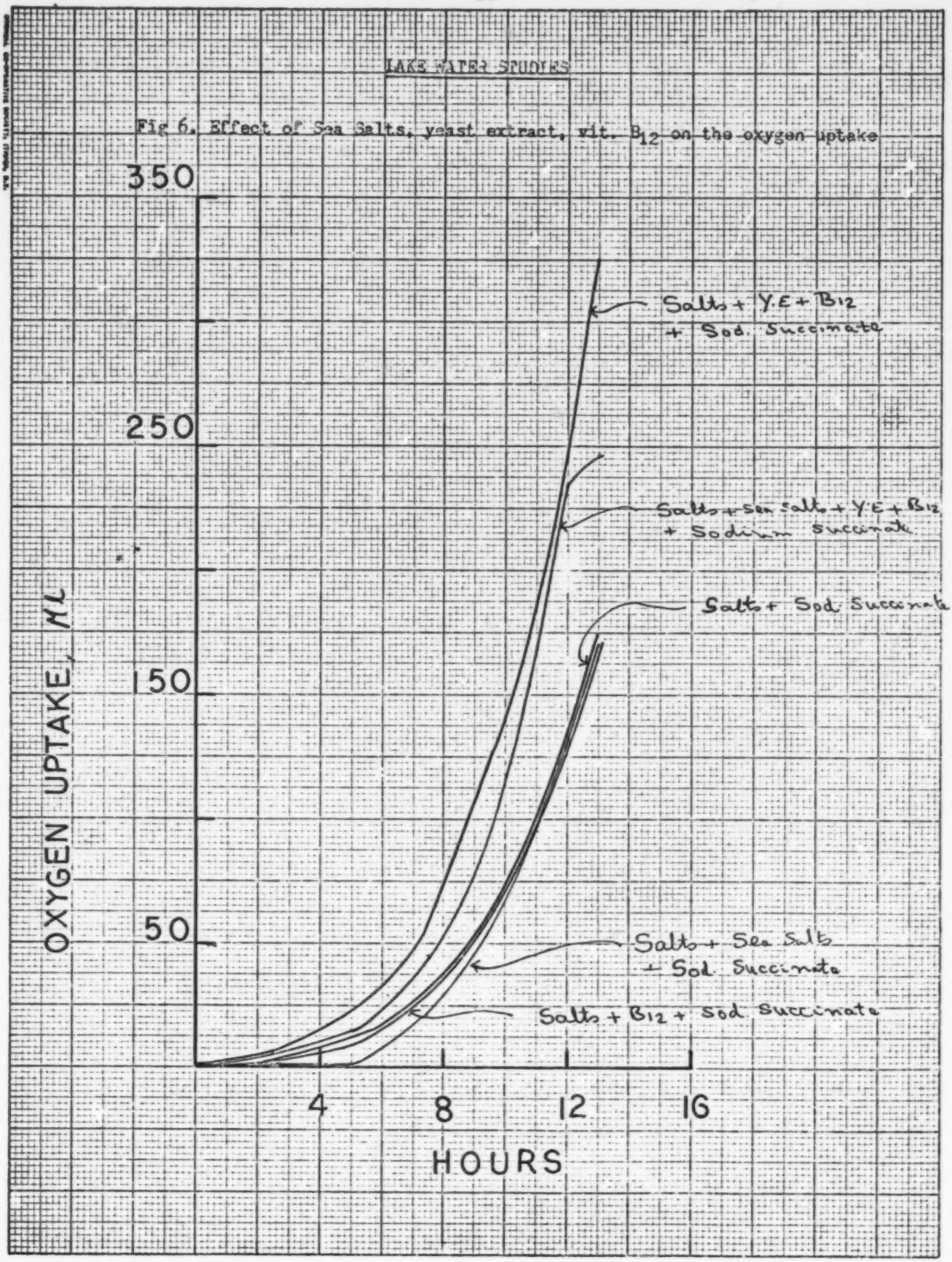
LAKE WATER STUDIES

Fig. 5. Effect of sea salts, yeast extract and vit. B_{12} on the Oxygen Uptake.



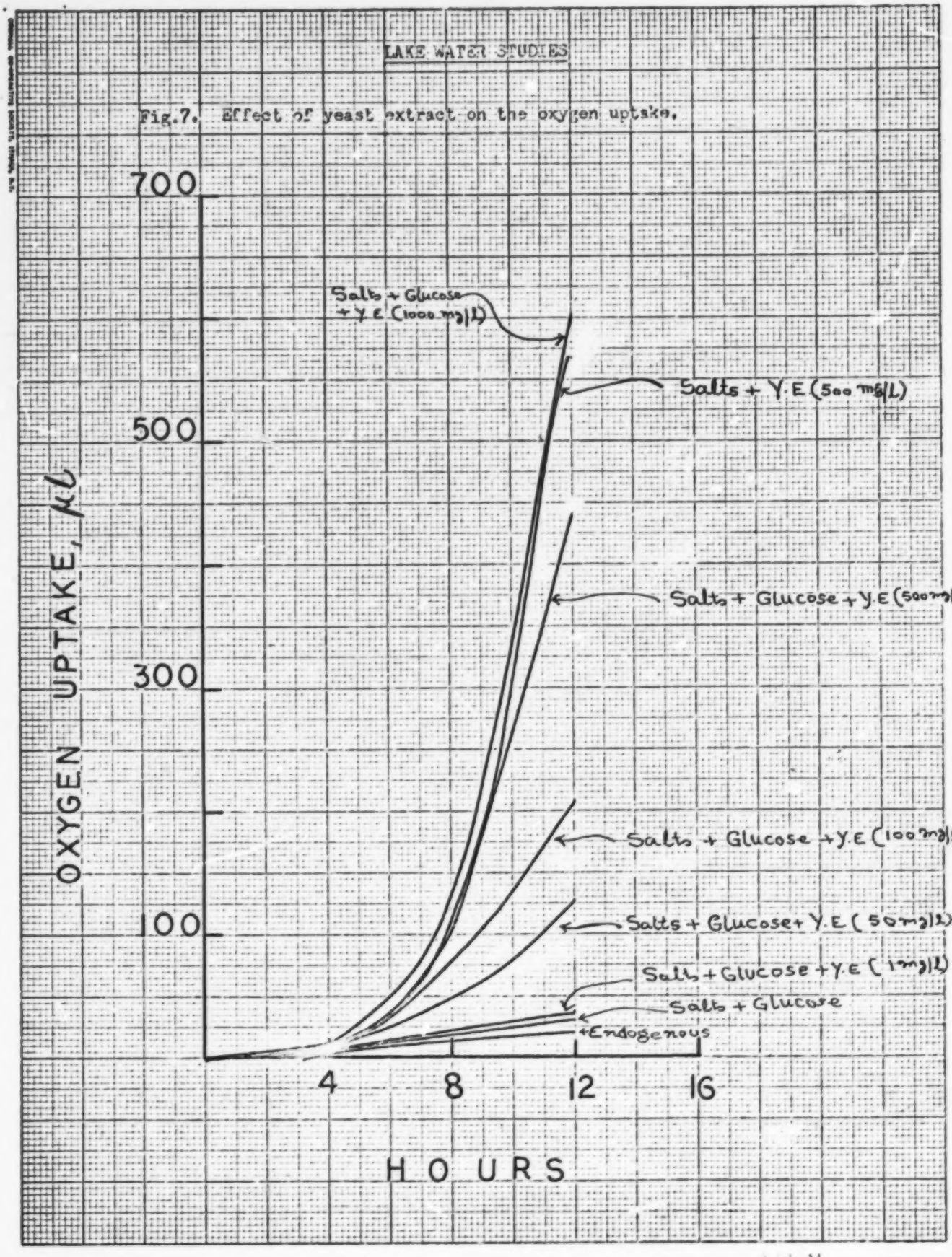
LAKE WATER STUDIES

Fig 6. Effect of Sea Salts, yeast extract, vit. B_{12} on the oxygen uptake



LAKE WATER STUDIES

Fig.7. Effect of yeast extract on the oxygen uptake.



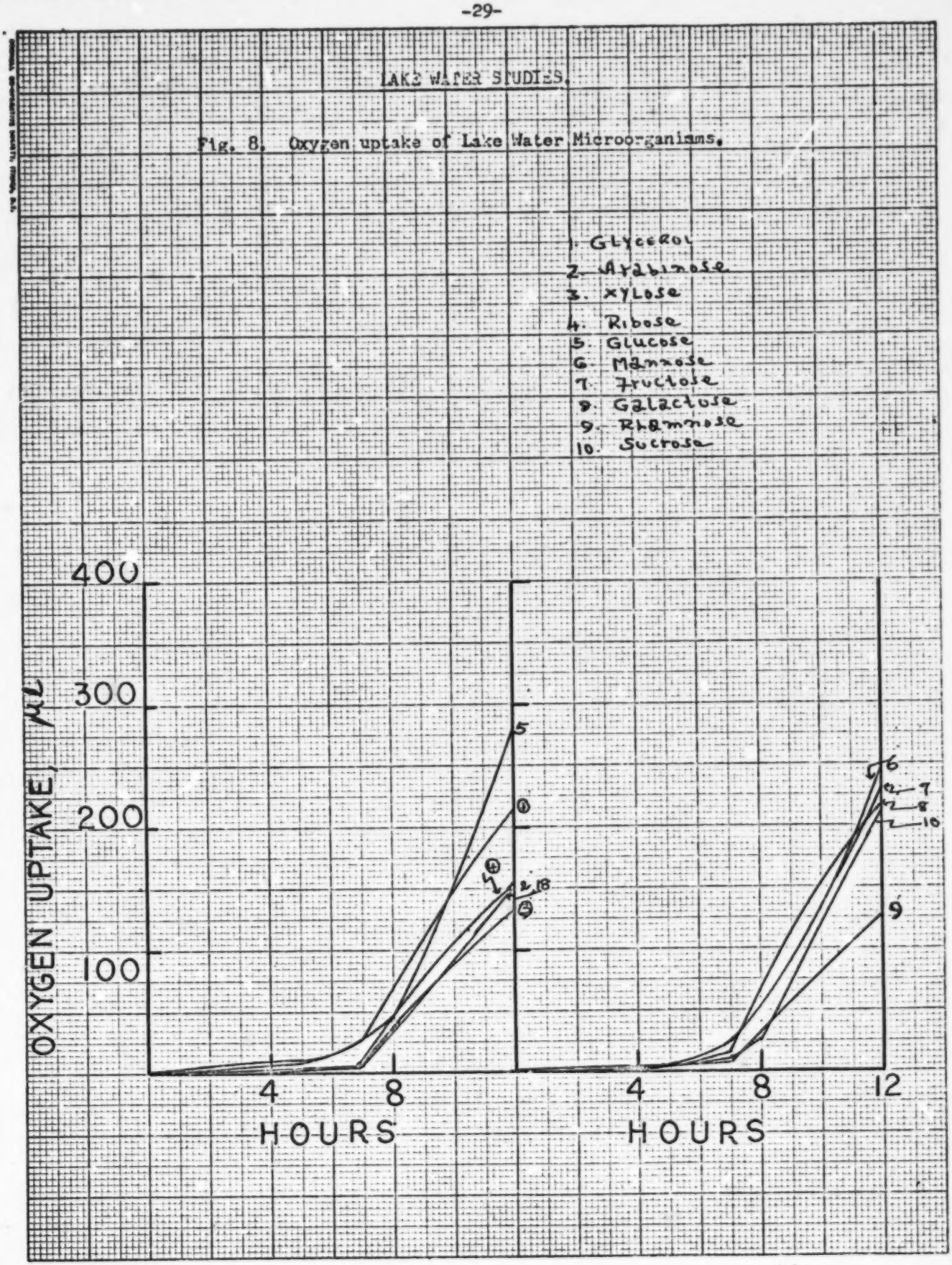
If the yeast extract were stimulatory rather than a substrate in itself, it would have elevated the oxygen uptake with the glucose even at very low levels, but as seen from the figures the O_2 uptake was more or less proportional to the amount of yeast extract present.

OXYGEN UPTAKE OF LAKE WATER COMPARED TO THAT OF TRICKLING FILTER MICROORGANISMS:

In comparing heterotrophy of the microbial populations of the lake water and trickling filters, oxygen uptake studies were undertaken using the previously listed substrates.

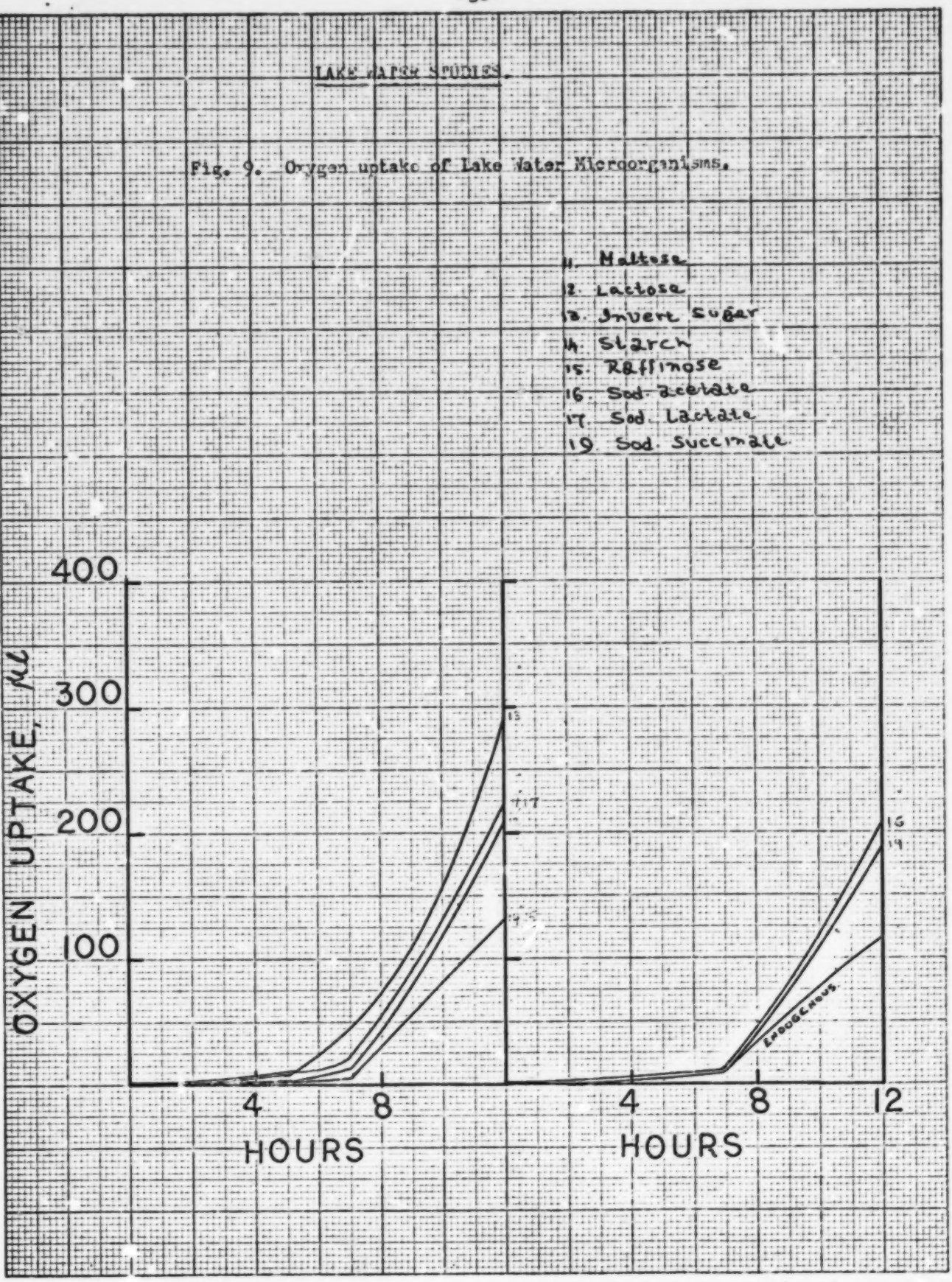
In these studies the suspended solids of the lake water and trickling filter inocula used were between 2500 to 3000 mg per l.

Figures 8, 9, 10 and 11 show the oxygen uptake patterns of the lake water population for the thirty-eight substrates tested. Figures 12, 13, 14 and 15 show the oxygen uptake pattern of the trickling filter microorganisms.


LAKE WATER STUDIES.
1. Glycerol
2. Arabinose
3. Xylose
4. Ribose
5. Glucose
6. Mannose
7. Fructose
8. Galactose
9. Rhamnose
10. Sucrose
OXYGEN UPTAKE, µL
400
300
200
100
4
8
HOURS
4
8
12
HOURS

LAKE WATER STUDIES.

Fig. 9. Oxygen uptake of Lake Water Microorganisms.



LAKE WATER STUDIES

Fig. 10. Oxygen Uptake of Lake Water Microorganisms

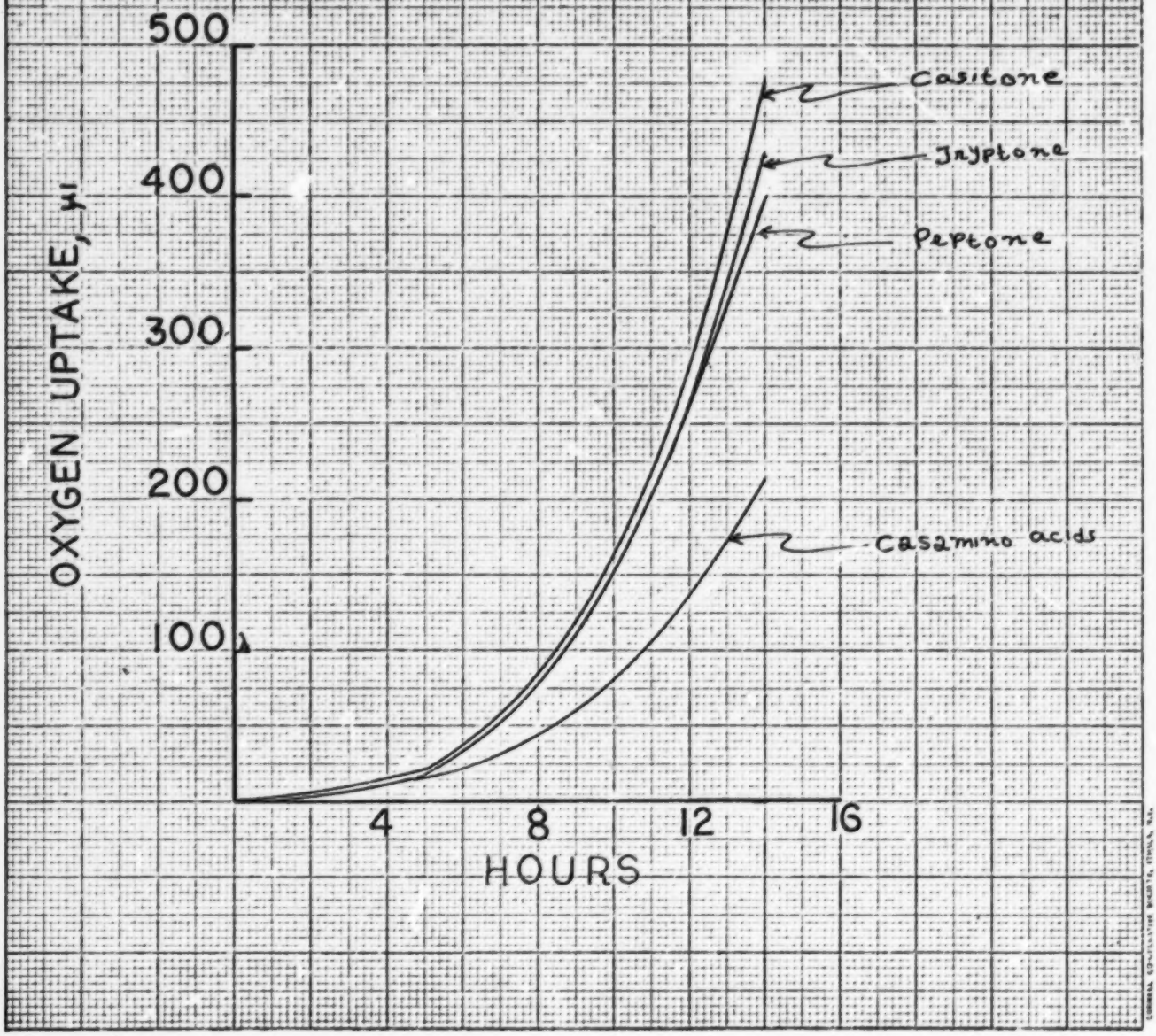


Fig. 11. Oxygen Uptake of Lake Water Microorganisms

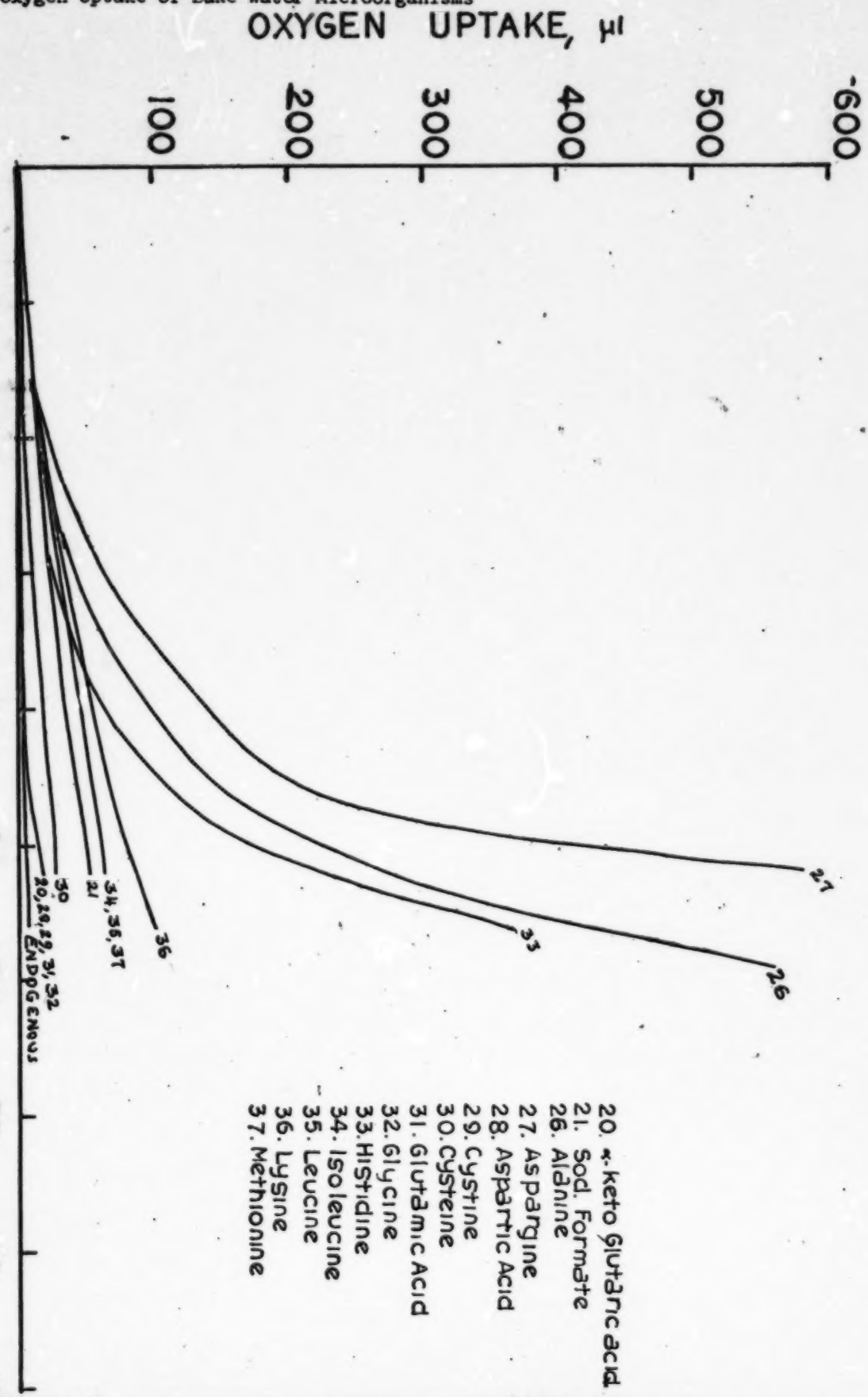


Fig. 12. Oxygen Uptake of Trickling Filter Flora.

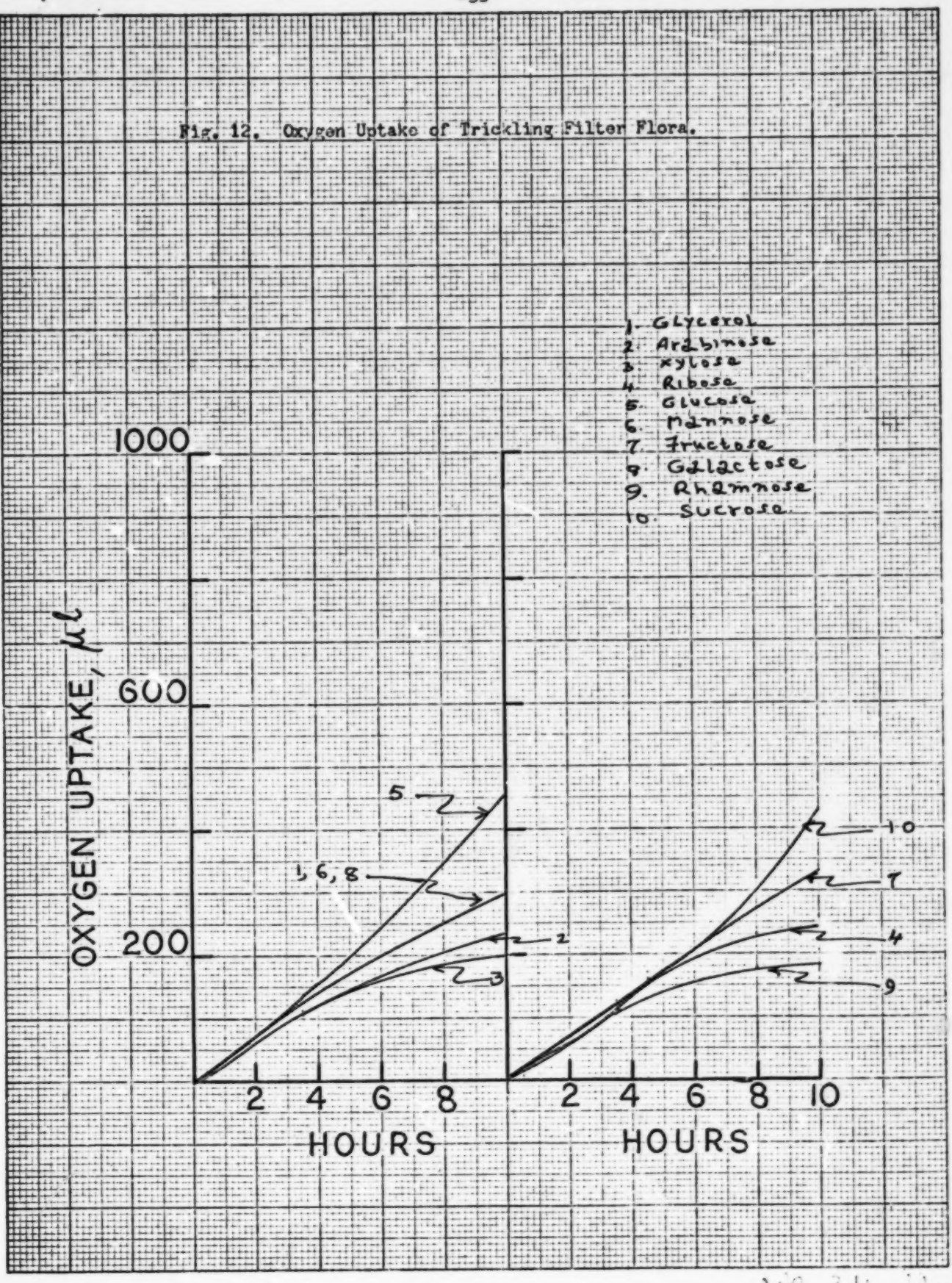


Fig. 13. Oxygen uptake of Trickling Filter Flora.

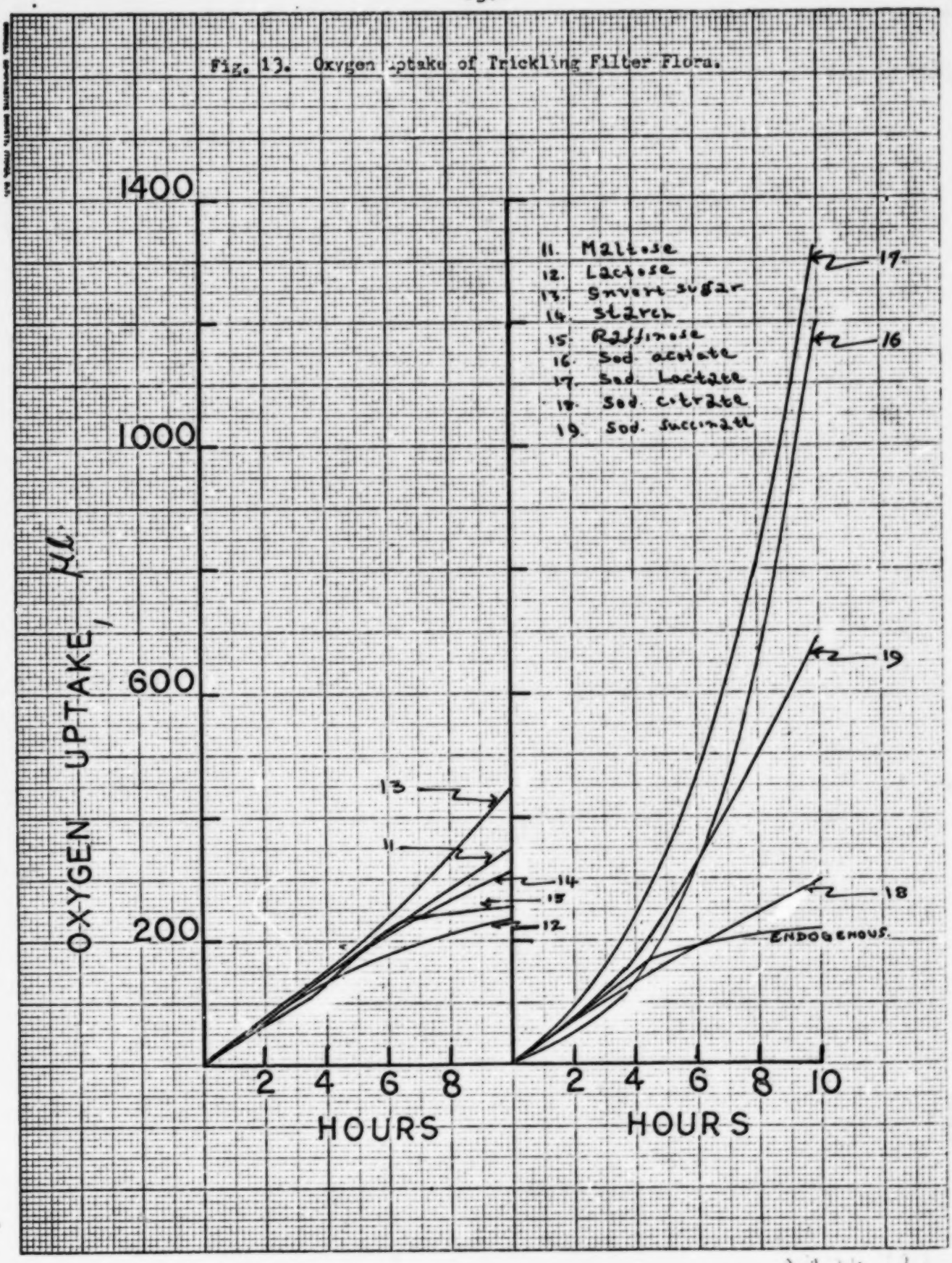


Fig. 14. Oxygen Uptake of Trickling Filter Flora

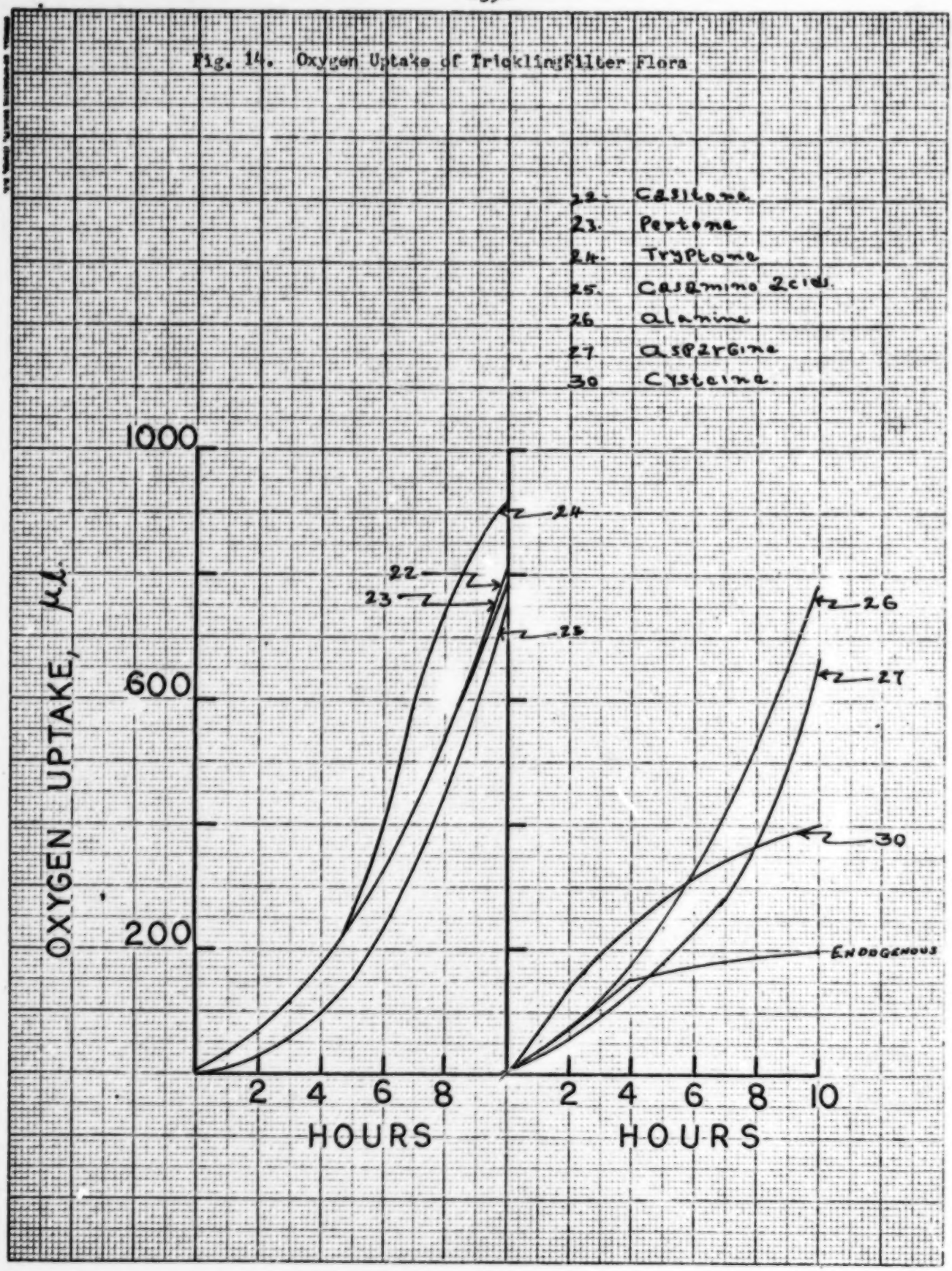
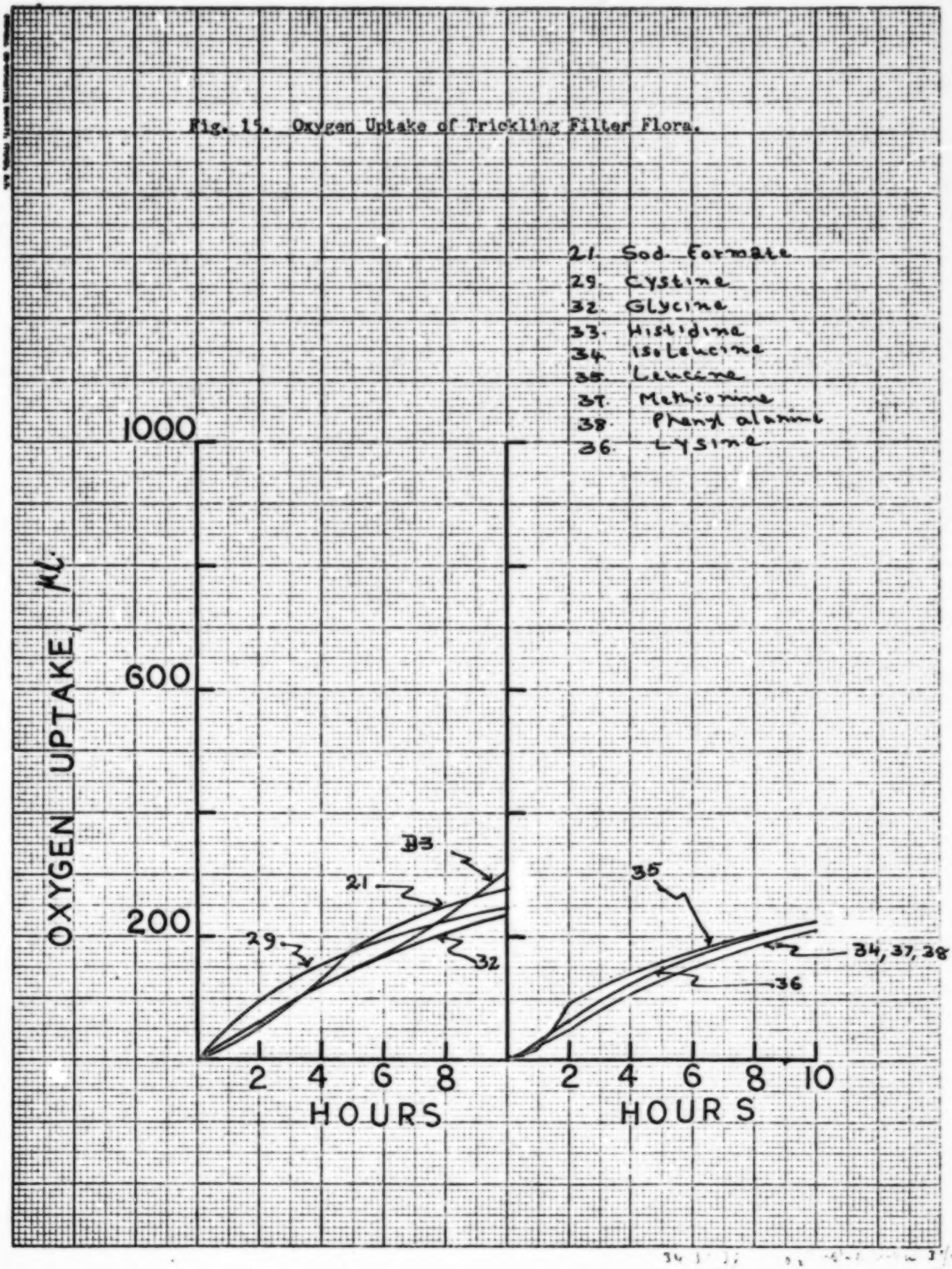


Fig. 15. Oxygen Uptake of Trickling Filter Flora.



On comparing the oxygen uptake patterns of the two populations, it can be seen that they are different. In general, the oxygen uptake of trickling filter populations is higher than the lake water populations for comparable periods. Although several reasons may be postulated for this, they have not yet been definitely ascertained.

The lake water populations exhibited lags for about 7 hours before exerting substantial oxygen uptake on glycerol, arabinose, invert sugar, xylose, ribose, glucose, mannose, fructose, galactose, rhamnose, sucrose, maltose, starch, raffinose, acetate, lactate, and succinate. Of the substrates in this series, casitone, tryptone and peptone caused maximum uptake, although with a lag of five hours. Alanine, asparagine and histidine also caused substantial oxygen uptake although with prolonged lag periods. Although the lake water populations attacked peptones, they were not able to metabolize many of the amino acids tested up to this point, as shown in figure 11. It will probably be fruitful to pursue amino acid testing more intensively.

From Figures 13 and 14 it can be seen that the trickling filter population earlier exerted a greater oxygen uptake on acetate, lactate, succinate, casitone, peptone, tryptone, casamino acids, alanine, asparagine, and cysteine. The rest of the substrates exerted relatively smaller uptakes than the ones mentioned above. However, the magnitude of these O_2 uptakes was higher than the corresponding ones for lake water populations. Within the limited data gathered so far, it can be said that the lake water flora is different from that of the trickling filter in its ability, as a population, to attack organic substrates.

The oxygen uptake studies are still in progress, and more substrates are being tested.

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